OMB Control Number: 2040-0246
Expiration Date: 07/31/02

Application for the Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act

Part 1. Laboratory Information

Laboratory Name:		
Address:		
City:	**State:**	**Zip:**
Contact Person:		
Title:		
Telephone:	**Fax:**	
Email address:		
Type of laboratory (circle one): Commercial Utility State Academic Other		
Was your laboratory ICR-approved for protozoa? ☐ Yes ☐ No		
Is your laboratory currently participating in the EPA PE Program? ☐ Yes ☐ No		
Number of field samples analyzed by your laboratory using Method 1622/1623:		
Number of spiked samples analyzed by your laboratory using Method 1622/23:		
Number of fields samples your laboratory can currently analyze per month using Method 1622/1623:	**Number of field samples your laboratory could potentially analyze per month using Method 1622/1623 during LT2:**	

Part 2. Method Information: Versions of Method 1622/1623 for which the lab is seeking evaluation

Method step	Method 1622 (*Cryptosporidium* only)	Method 1623 (*Cryptosporidium* & *Giardia*)
Filtration (check all that apply and indicate the volume filtered for each)		
Gelman Envirochek		
Gelman HV Envirochek		
IDEXX FiltaMax		
Whatman CrypTest		
Other (describe)		
Elution (check all that apply)		
Wrist action shaker (Envirochek)		
Stomaching of FiltaMax filter		
FiltaMax wash station plunger		
Back Wash/Sonication (CrypTest)		
Other (describe)		
Concentration (check all that apply)		
Centrifugation		
Filtration through membrane		
Other (describe)		
Purification (check all that apply)		
Dynal anti-Crypto, Dynal GC-combo		
Other (describe)		
Staining (check all that apply)		
Waterborne AquaGlo		
Waterborne Crypt-a-Glo		
Waterborne Giardi-a-Glo		
Meridian Merifluor		
Other (describe)		
Descriptions of "other" method steps and other comments:		

Part 3. Personnel Information (attach additional sheets if necessary)

1. Principal Analyst/Supervisor : one required per approved laboratory			
Name		**Current position**	
Academic training/degree(s)		**Time in current position**	
No. of samples processed for protozoa analyses		**No. of samples processed using Methods 1622/1623**	
Was this person approved as an analyst under the ICR? ☐ **Yes** ☐ **No**			
Portions of method currently performed (circle all that apply): Filtration Elution Concentration IMS Staining Examination			
2. Analyst or Technician (circle one)			
Name		**Current position**	
Academic training/degree(s)		**Time in current position**	
No. of samples processed for protozoa analyses		**No. of samples processed using Methods 1622/1623**	
Was this person approved under the ICR? ☐ **Yes** ☐ **No** **If yes then check one:** ☐ **Analyst** ☐ **Technician**			
Portions of method currently performed (circle all that apply): Filtration Elution Concentration IMS Staining Examination			
3. Analyst or Technician (circle one)			
Name		**Current position**	
Academic training/degree(s)		**Time in current position**	
No. of samples processed for protozoa analyses		**No. of samples processed using Methods 1622/1623**	
Was this person approved under the ICR? ☐ **Yes** ☐ **No** **If yes then check one:** ☐ **Analyst** ☐ **Technician**			
Portions of method currently performed (circle all that apply): Filtration Elution Concentration IMS Staining Examination			
4. Analyst or Technician (circle one)			
Name		**Current position**	
Academic training/degree(s)		**Time in current position**	
No. of samples processed for protozoa analyses		**No. of samples processed using Methods 1622/1623**	
Was this person approved under the ICR? ☐ **Yes** ☐ **No** **If yes then check one:** ☐ **Analyst** ☐ **Technician**			
Portions of method currently performed (circle all that apply): Filtration Elution Concentration IMS Staining Examination			
5. Analyst or Technician (circle one)			
Name		**Current position**	
Academic training/degree(s)		**Time in current position**	
No. of samples processed for protozoa analyses		**No. of samples processed using Methods 1622/1623**	
Was this person approved under the ICR? ☐ **Yes** ☐ **No** **If yes then check one:** ☐ **Analyst** ☐ **Technician**			
Portions of method currently performed (circle all that apply): Filtration Elution Concentration IMS Staining Examination			

Part 4. Laboratory Equipment Confirmation Checklist for Methods 1622 and 1623

Key Equipment and Reagents	Manufacturer/Model	If not Present, Proof of Purchase Attached (Y/N)
Filtration and elution		
Flow control valve - 0.5 gpm		
Centrifugal or other pump		
Low-flow meter or graduated container		
Laboratory shaker for agitating capsule filters (Envirochek only)		
Laboratory shaker side arms (Envirochek only)		
Filter housing (CrypTest or Filta-Max)		
Wash station (Filta-Max only)		
Stomacher (Filta-Max only)		
Compressed air source (CrypTest only)		
Sonicator (CrypTest only)		
Concentration		
Concentrator apparatus (Filta-Max only)		
1500 X G, swinging-bucket centrifuge for 15 mL - 250-mL tubes		
Immunomagnetic separation		
Sample mixer/rotator for 10-mL tubes		
Magnetic particle concentrator for 10-mL tubes		
Magnetic particle concentrator for 1.5-mL tubes		
Flat-sided sample tubes		
Examination		
Epifluorescence/differential interference contrast microscope with stage and ocular micrometers and 20X to 100X objectives		
Excitation/band pass microscope filters for fluorescein isothiocyanate (FITC) assay		
Excitation/band-pass filters for 4',6-diamidino-2-phenylindole (DAPI) assay		

The above application information is complete and accurate to the best of my knowledge.

_____ _____

Name and Signature Laboratory Manager or Designee **Date**

Submit application package to: **Jennifer Scheller, *Cryptosporidium* Laboratory Quality Assurance Evaluation Program Coordinator, DynCorp I&ET, 6101 Stevenson Avenue, Suite 500, Alexandria, VA 22304**

Audit Checklist (To Be Used for Self-Audit)

Part A: Facilities, Equipment, and Quality Assurance

Item to be Evaluated	Classification	Yes, No, Unknown*, or NA
1 Laboratory Equipment and Supplies		
1.1 Reagent-grade water testing		
1.1.1 Is reagent water tested monthly for these minimum parameters: conductivity, total chlorine residual; and annually for metals-Pb, Cd, Cr, Cu, Ni, Zn?	Requirement	
1.1.2 Were the results for the above parameters acceptable, total chlorine residual not greater than 0.1 mg/L, conductivity not greater than 2 µmhos/cm, and each metal not greater than 0.05 mg/L and collectively not greater than 0.1 mg/L?	Requirement	
1.1.3 Is reagent water tested monthly for heterotrophic plate count?	Requirement	
1.1.4 Are the results for the heterotrophic plate count acceptable, < 500/mL?	Requirement	
1.2 Laboratory pH meter:		
1.2.1 Accuracy ± 0.1 units, scale graduations, 0.1 units?	Requirement	
1.2.2 Is a record maintained for pH measurements and calibrations used?	Requirement	
1.2.3 Is pH meter standardized each use period with pH 7, 4 or 10 standard buffers (selection dependant upon desired pH)?	Requirement	
1.2.4 All pH buffers are dated when received and opened and are discarded before expiration date?	Requirement	
1.3 Balances (top loader or pan balance):		
1.3.1 Are balances calibrated monthly using Class S/S-1 weights, or weights traceable to Class S/S-1 weights?	Requirement	
1.3.2 Is correction data available with S/S-1 weights?	Requirement	
1.3.3 Is preventative maintenance conducted yearly at a minimum?	Recommendation	
1.4 Autoclave:		
1.4.1 Is unit equipped with a temperature gauge/operational safety valve?	Requirement	
1.4.2 Are date, contents, sterilization time and temperature recorded for each cycle?	Requirement	
1.4.3 Is a maximum registering thermometer or continuous monitoring device used during each autoclave cycle?	Requirement	
1.4.4 Is automatic timing mechanism checked with stopwatch quarterly?	Requirement	
1.4.5 Are spore strips or ampules used monthly to confirm sterilization?	Requirement	
1.5 Refrigerator/Freezer:		
1.5.1 Is refrigerator able to maintain temperature of 1°C to 5° C?	Requirement	
1.5.2 Is temperature recorded once daily for days in use?	Requirement	
1.6 Temperature recording device:		
1.6.1 Are calibration of glass/mercury thermometers checked annually (dial thermometers quarterly) at the temperature used against a reference NIST thermometer or equivalent?	Requirement	
1.7 Micropipetters:		
1.7.1 Have micropipetters been calibrated within the past year? [Section 9.2.1]	Requirement	
1.8 Centrifuge		
1.8.1 Is a maintenance contract in place, or internal maintenance protocol available?	Requirement	
1.8.2 Is RPM and RCF calibrated yearly?	Requirement	
1.9 General		
1.9.1 Are calibration and maintenance records complete and well organized?	Recommendation	

Item to be Evaluated	Classification	Yes, No, Unknown*, or NA
2 Quality Assurance		
2.1 Does the laboratory have a formal QA laboratory plan prepared and ready for examination?	Requirement	
2.2 Are employee resumes present and complete?	Requirement	
2.3 Is a training protocol for new employees present?	Recommendation	
2.4 Is the laboratory performing analyst verification of examination monthly and does the lab have corrective action procedures in place if criteria are not met? (Section 10.5)	Requirement	
2.5 Are employee training records available and up to date?	Requirement	
2.5.1 Have technicians/analysts analyzed the required number of samples using Method 1622/1623?	Requirement	
2.6 Are all relevant SOPs present and current?	Requirement	
2.7 Are sampling instructions present for clients collecting and/or filtering samples in the field?	Requirement	
2.8 Does the laboratory have criteria for sample acceptance and corrective action procedures?	Requirement	
2.9 Are data recording procedures present?	Requirement	
2.9.1 Does the laboratory have an SOP for checking all manual calculations?	Requirement	
2.10 Are corrective action contingencies present?	Requirement	
2.10.1 For OPR failures? [Section 9.7.4]	Requirement	
2.10.2 For method blank contamination?	Requirement	
2.10.3 For positive/negative staining control failures?	Requirement	
2.11 Does the quality assurance plan specifically address requirements for protozoa analysis under the programs for which the laboratory intends to analyze samples?	Requirement	
2.12 Is a laboratory organization chart or other information available listing staff organization and responsibilities? Does it identify the QA manager?	Requirement	
2.12.1 Is the QA manager separate from the lab manager?	Recommendation	
2.13 Does the laboratory have a list of preventative maintenance procedures and schedules?	Requirement	
2.14 Date range covered for quality control (QC) sample audit?		
2.15 When did the laboratory begin processing samples with the Envirochek filter?		/ /
2.16 When did the laboratory begin processing samples with the Filta-Max filter (if applicable)?		/ /
2.17 When did the laboratory begin processing samples with the CrypTest filter (if applicable)?		/ /
2.18 Approximately how many field samples were analyzed using methods 1622/1623 since the lab started using Method 1622/1623?		Field samples ___ MS_____
2.19 Have acceptable initial precision and recovery analyses been performed for each version of the method the laboratory is using?	Requirement	
2.20 Were method blanks run once per week or per 20 samples during this period? [Section 9.6.1]	Requirement	
2.20.1 If the answer to 2.20 is no, then at what frequency where method blanks performed?		
2.20.2 What percentage of method blanks evaluated were without contamination?		
2.20.3 Was an acceptable method blank associated with each field sample examined?	Requirement	
2.20.4 How many method blanks were evaluated?		
2.21 Were ongoing precision and recovery (OPR) samples run once per week or per 20 samples during this period? [Section 9.7]	Requirement	
2.21.1 If the answer to 2.21 is no, then at what frequency where OPR samples performed?		
2.21.2 What percentage of OPR samples evaluated met the recovery criteria? [Table 3; Section 9.7.3]		

Item to be Evaluated	Classification	Yes, No, Unknown*, or NA
2.21.3 Does the laboratory maintain control charts of OPR results? [Section 9.7.5]	Requirement	
2.21.4 Was an acceptable OPR associated with each field sample examined?	Requirement	
2.21.5 How many OPR samples were evaluated?		
2.21.6 How many OPR samples were analyzed during the past six months?		
2.21.7 What is the mean and relative standard deviation of the recoveries of the OPR samples analyzed during the past six months?		Mean_____ RSD_____
2.22 Were matrix spike (MS) samples analyzed at the method -specified frequency? [Section 9.1.8]	Requirement	
2.22.1 If the answer to 2.22 is no, then at what frequency were MS samples analyzed?		
2.22.2 How many MS samples were evaluated?		
2.22.3 How many MS samples were analyzed during the past six months?		
2.22.4 What is the mean and relative standard deviation of the MS samples analyzed during the past six months?		Mean_____ RSD_____
2.23 Were OPR and MS samples spiked with 100 - 500 organisms? [Section 9.7]	Requirement	
2.23.1 If the answer to 2.23 is no, then at what level were samples spiked?		
2.24 Are the laboratory personnel performing the QC analyses representative of the personnel seeking approval under this program?	Requirement	
2.25 Does the laboratory have records of all QC checks available for inspection?	Requirement	
2.26 Does the laboratory have an adequate record system for tracking samples from collection through log-in, analysis, and data reporting?	Requirement	
2.27 Are results from each sample maintained electronically?		
2.28 If data are stored electronically, are files backed up on more than one disk to ensure data are not lost in the eventuality of some hardware failure?	Requirement	
2.29 If data is stored electronically, does the laboratory have an SOP for checking the accuracy of data entry into an electronic system?	Requirement	
2.30 Is the laboratory using the April 2001 version of Method 1622/1623?	Requirement	
3 Data Recording Procedures		
3.1 Is shipping information complete, including the time and date of sample receipt, sample condition, and noting any discrepancies between samples on the traffic report and samples received?	Requirement	
3.2 Do sample numbers on the shipping forms match the sample numbers on the report forms?	Requirement	
3.3 Are current Method 1622/1623 bench sheets used to record sample processing data?	Recommendation	
3.4 Are all primary measurements during each step recorded, including all raw data used in calculations?	Requirement	
3.5 Name of analyst or technician performing the elution is recorded?	Requirement	
3.6 Date and time of elution is recorded?	Requirement	
3.7 Name of analyst or technician performing the concentration is recorded?	Requirement	
3.8 Date and time of concentration is recorded?	Requirement	
3.9 Are batch and lot numbers of reagents used in the analysis of the sample recorded?	Requirement	
3.10 Lot number for the IMS kit is recorded?	Requirement	
3.11 Are Method 1622/1623 *Cryptosporidium* report forms used to record sample examination results?	Requirement	
3.12 Name of examining analyst is recorded?	Requirement	
3.13 Date and time of sample examination is recorded?	Requirement	
3.14 Are calculations of final concentrations and recoveries complete and correct?	Requirement	

Item to be Evaluated	Classification	Yes, No, Unknown*, or NA
3.15 Do values recorded on the data sheets match the reported values?	Requirement	
3.16 Are mistakes on all forms crossed out with a single line, initialed, and dated?	Requirement	
3.17 Are data always recorded in pen?	Requirement	
3.18 Are hardcopy records well organized, complete, and easily accessible?	Requirement	
3.19 Does the laboratory include a disclaimer on the report to the client if method QC requirements were not met?	Recommendation	
3.20 Is the manually recorded data legible?	Requirement	
3.21 Do records demonstrate each analyst's characterization of 3 oocysts and 3 cysts from positive control for each microscopy session? [Section 15.2.1.1]	Requirement	
3.22 Data shows that no more than 0.5 mL of pellet was used per IMS? [Section 13.2.4]	Requirement	
4 Holding Times		
4.1 Samples analyzed according to December 1999 version of Method 1622/1623		
4.1.1 Is time from initiation of sample collection to completion of concentration 72 hours or less? [Section 8.1]	Requirement	
4.1.2 Concentrate is held no longer than 24 hours between IMS and staining? [Section 8.2]	Requirement	
4.1.3 Are stained slides read and confirmed within 72 hours of staining? [Section 8.4]	Requirement	
4.2 Samples analyzed according to April 2001 version of Method 1622/1623		
4.2.1 Is sample elution initiated within 96 hours of sample collection or field filtration? [Section 8.2.1]	Requirement	
4.2.2 Are sample elution, concentration, and purification steps completed in one work day? [Section 8.2.2]	Requirement	
4.2.3 Are slides stained within 72 hours of application of the purified sample to the slide? [Section 8.2.3]	Requirement	
4.2.4 Are stained slides read and confirmed within 7 days of staining? [Section 8.2.4]	Requirement	
5 Spike enumeration procedures		
5.1 What method does the laboratory currently use to estimate spike doses:(A) flow-sorted spikes, (B) well-slide-counted spikes, (C) hemacytometer-counted spikes, or (D) membrane-filter-counted spikes		Circle one: A B C D
5.1.1 If flow-sorted spikes are used, on what date did the laboratory begin using flow-sorted spikes?		/ /
5.1.2 If counted manually, does the laboratory follow Method 1622/1623 procedures for establishing spike level? [Section 11.3]	Requirement	
5.1.3 What were the relative standard deviations of the last four spike enumerations?		1.
		2.
		3.
		4.
5.2 Source of oocysts for spikes		
5.3 If 50-L samples are analyzed, what positive control procedure does the laboratory follow for OPR and MS samples: (A) spike entire 50 L, (B) spike and filter 10 L before filtering 40 L, or (C) filter 40 L before spiking and filtering 10 L.		

*Unknown response requires an explanation

Note: All section references in [ ] refer to Method 1623 April 2001

Part B: Sample Processing and Examination

Item to be evaluated		Classification	Yes, No, NA or Unknown
6	**Laboratory Facilities and Laboratory Safety**		
6.1	Are laboratory coats and gloves worn in the laboratory?	Requirement	
6.2	No other safety or facility issues were observed?		
7	**Sample Spiking** **Technician:**		
7.1	What method does laboratory currently use to estimate spike doses:(A) flow-sorted spikes, (B) well-slide-counted spikes, (C) hemacytometer-counted spikes, or (D) membrane-filter-counted spikes		Circle one: A B C D
7.2	With what filter type did the laboratory demonstrate their spiking procedure?		
7.3	Is the carboy used for negative control randomly selected from carboy stock to check efficacy of cleaning system?	Requirement	
7.4	If flow-sorted spikes are used, was suspension vial vortexed for two minutes or per manufacturers instructions? [Section 11.4.3]	Method Procedure	
7.5	Was the suspension vial adequately rinsed? [Section 11.4.3.1]	Method Procedure	
7.6	Does the laboratory have an acceptable SOP for sample spiking?	Requirement	
7.7	Other than the issues noted for items 7.2 through 7.6 (if any) was sample spiking demonstrated successfully?		
8	**Envirochek (Complete Sections that apply)**		
8.1	**Envirochek Filtration** **Technician:**		
8.1.1	Are all components required for sample filtration present and in good condition? [Section 6.2]	Requirement	
8.1.2	Is the filter assembly set up correctly? [Figure 3, pg 48]	Method Procedure	
8.1.3	Is the pump adequate for needs? [Section 6.3.3]	Requirement	
8.1.4	Is the appropriate flow rate maintained (approximately 2L/min)? [Section 12.2.1.2]	Method Procedure	
8.1.5	Is the volume filtered measured using a flow meter or calibrated carboy? [Section 12.2.4.2]	Requirement	
8.1.6	Is the system well maintained and cleaned appropriately following use?	Requirement	
8.1.7	Is the system able to maintain seal during use with no leaks?	Requirement	
8.1.8	Does the laboratory have an acceptable SOP for Envirochek filtration?	Requirement	
8.1.9	Other than the issues noted in items 8.1.1 through 8.1.8, was Envirochek filtration demonstrated successfully?		
8.2	**Envirochek capsule filter elution** **Technician:**		
8.2.1	Is the elution buffer prepared as per Method 1622/1623? [Section 7.4]	Method Procedure	
8.2.2	Is the wrist-shaker assembly set up correctly? [Section 12.2.6.1.1]	Method Procedure	
8.2.3	Does the eluting solution cover the membrane? [Section 12.2.6.2.2]	Method Procedure	
8.2.4	Are the samples shaken at an appropriate speed? [Section 12.2.6.2.3]	Method Procedure	
8.2.5	Are the samples shaken three times for 5 minutes each time, and each in a different orientation? [Section 12.2.6.2]	Method Procedure	
8.2.6	Does the laboratory have an acceptable SOP for Envirochek capsule filter elution?	Requirement	
8.2.7	Other than the issues noted for items 8.2.1 through 8.2.7 (if any) was Envirochek filter elution demonstrated successfully?		
9	**CrypTest**		
9.1	**CrypTest Filtration** **Technician:**		
9.1.1	Are all components required for sample filtration present and in good condition? [Section 6.2.3]	Requirement	

Item to be evaluated		Classification	Yes, No, NA or Unknown
9.1.2	Is the filter assembly set up correctly?	Method Procedure	
9.1.3	Is the pump adequate for needs? [Section 6.3.3]	Requirement	
9.1.4	Is the appropriate flow rate maintained (approximately 2L/min)?	Method Procedure	
9.1.5	Is the volume filtered measured using a flow meter or a calibrated carboy?	Requirement	
9.1.6	Is the system well maintained and cleaned appropriately following use?	Requirement	
9.1.7	Is the system able to maintain seal during use with no leaks?	Requirement	
9.1.8	Does the laboratory have an acceptable SOP for CrypTest Filtration?	Requirement	
9.1.9	Other than the issues noted in items 9.1.3 through 9.1.10 (if any) was CrypTest filtration demonstrated successfully?		
9.2 CrypTest cartridge filter elution	**Technician:**		
9.2.1	Does the filter seat properly in the filter housing, so there are no leaks?	Requirement	
9.2.2	Is the elution buffer prepared according to manufacturer's instructions? [Section 7.4.2]	Method Procedure	
9.2.3	Is an appropriate amount of elution solution backwashed into the filter housing? (approx. 150 mL)	Method Procedure	
9.2.4	Is the assembly well sealed (no leaks)?	Requirement	
9.2.5	Is sonication performed for 2 minutes?	Method Procedure	
9.2.6	Is the filter elution repeated, according to the manufacturer's instructions?	Method Procedure	
9.2.7	Following the last elution, is the remaining elution buffer driven from the outlet side to the inlet side and into the sample bottle?	Requirement	
9.2.7.1	Is the regulated compressed air source used, sufficient to drive the eluting buffer from the filter?	Requirement	
9.2.8	After elution is complete, is the filter removed from the housing and the base, lid, and lip of the filter housing rinsed using eluting solution and added to the sample bottle?	Requirement	
9.2.9	Does the laboratory have an acceptable SOP for CrypTest elution?	Requirement	
9.2.10	Other than the issues noted in items 9.2.1 through 9.2.9 (if any) was CrypTest filter elution demonstrated successfully?		
10 Filta-Max			
10.1 Filta-Max filtration	**Technician:**		
10.1.1	Are all components required for sample filtration present and in good condition? [Section 6.2.4]	Requirement	
10.1.2	Is the filter assembly set up correctly?	Method Procedure	
10.1.3	Is appropriate flow rate maintained of <4 L per minute?	Method Procedure	
10.1.4	Is the volume filtered measured correctly using a flow meter or calibrated carboy?	Requirement	
10.1.5	Is system well maintained and cleaned appropriately following use?	Requirement	
10.1.6	Is system able to maintain seal during use with no leaks?	Requirement	
10.1.7	Does the laboratory have an acceptable SOP for Filta-Max filtration?	Requirement	
10.1.8	Does the laboratory indicate on the filter housing the correct direction of flow?	Requirement	
10.1.9	Other than the issues noted in items 10.1.1 through 10.1.8 (if any) was Filta-Max filtration demonstrated successfully?		
10.2 Filta-Max filter wash station elution	**Technician:**		
10.2.1	Is an automatic or manual wash station used?		
10.2.2	Is the filter wash station set up correctly?	Requirement	
10.2.3	Is PBST used to elute the filter? [Section 7.4.2]	Method Procedure	

Item to be evaluated	Classification	Yes, No, NA or Unknown
10.2.4 Is an appropriate amount of PBST used for each wash? (approx. 600 mL)	Method Procedure	
10.2.5 During the first wash, is the plunger moved up and down 20 times?	Method Procedure	
10.2.6 Is the plunger moved up and down gently to avoid generating excess foam?	Method Procedure	
10.2.7 During the second wash, is the plunger moved up and down 10 times?	Method Procedure	
10.2.8 If the automatic washer is used, is the machine operating properly?	Requirement	
10.2.9 Is the wash station cleaned adequately between samples?	Requirement	
10.2.10 Does the laboratory have an acceptable SOP for Filta-Max elution with the wash station?	Requirement	
10.2.11 Other than the issues noted for items 10.2.2 through 10.2.10 (if any) was elution of the Filta-max filter using the wash station demonstrated successfully?		
10.3 Filta-Max filter stomacher elution Technician		
10.3.1 Is PBST used to elute the filter? [Section 7.4.3.4]	Method Procedure	
10.3.2 Is an appropriate amount of PBST used for each wash? (approx. 600 mL)	Method Procedure	
10.3.3 Are two washes performed for 5 minutes each?	Method Procedure	
10.3.4 Is the stomacher in good condition and operating properly?	Requirement	
10.3.5 Does the laboratory have an acceptable SOP for Filta-Max elution using a stomacher?	Requirement	
10.3.6 Other than the issues noted for items 10.3.1 through 10.3.5 (if any) was elution of the Filta-Max filter using the stomacher demonstrated successfully?		
10.4 Filta-Max filter sample concentration (as an alternative to Section 11) Technician:		
10.4.1 Is concentrator set up correctly?	Requirement	
10.4.2 Is the force of the vacuum maintained below 30 cm Hg?	Method Procedure	
10.4.3 Is concentration performed after each of the washes?	Method Procedure	
10.4.4 Is the concentrate from the first wash added to the 600mL of eluate from the second wash?	Method Procedure	
10.4.5 Is the sample concentrated so that some liquid remains above the filter (enough to cover the stirbar about half-way)?	Method Procedure	
10.4.6 Is the stir bar and concentration tube rinsed after each concentration and the liquid added to the concentrate?	Requirement	
10.4.7 Was the filter membrane washed twice?	Method Procedure	
10.4.8 Was 5 mL of PBST used each time?	Method Procedure	
10.4.9 Is the membrane adequately washed to remove oocysts from filter?	Method Procedure	
10.4.10 Is the pellet volume determined?	Requirement	
10.4.11 Is there a set of standards for comparison of pellet size?	Recommendation	
10.4.12 Does the laboratory have an acceptable SOP for concentration using the Filta-Max concentrator?	Requirement	
10.4.13 Other than the issues noted in items 10.4.1 through 10.4.12 (if any) was sample concentration using the Filta-Max concentrator demonstrated successfully?		
11 Concentration		
11.1 Envirochek, CrypTest, and Filta-Max filter sample centrifugation Technician:		
11.1.1 Is the sample centrifuged at 1500 x G using a swinging bucket rotor? [Section 13.2.1]	Method Procedure	
11.1.2 Are the centrifuge tubes properly balanced prior to centrifugation?	Requirement	
11.1.3 Is the sample centrifuged for 15 minutes? [Section 13.2.1]	Method Procedure	
11.1.4 Is the centrifuge slowly decelerated at the end without the brake? [Section 13.2.1]	Method Procedure	
11.1.5 Is the pellet volume determined?	Requirement	

Item to be evaluated	Classification	Yes, No, NA or Unknown
11.1.6 Is there a set of standards for comparison of pellet size?	Recommendation	
11.1.7 Does the laboratory have an acceptable SOP for sample concentration?	Requirement	
11.1.8 Is residual suspension rinsed from all containers and gloves?	Requirement	
11.1.9 Other than the issues noted in items 11.1.1 through 11.1.8 (if any) was sample concentration demonstrated successfully?		
12 Reagents, equipment and clean-up		
12.1 Source for reagent-grade water:		
12.1.1 Is still or DI unit maintained according to manufacturer's instructions?	Requirement	
12.1.2 Is reagent grade water used to prepare all media and reagents? [Section 7.3]	Requirement	
12.2 Centrifuge:		
12.2.1 Does centrifuge have a swinging bucket rotor? [Section 6.8.1]	Requirement	
12.2.2 Is the centrifugation nomograph for determining relative centrifugal force located close to the centrifuge(s)?	Requirement	
12.3 SOP's for Reagents		
12.3.1 Are SOP's available for the preparation of all essential chemicals and reagents?	Requirement	
12.3.2 Are SOP's posted or easily accessible at the bench?	Recommendation	
12.3.3 Are all reagents clearly labeled with date of preparation, technician initials, and expiration date?	Requirement	
12.4 Clean-up		
12.4.1 Is all glassware and plasticware washed well and stored appropriately between uses?	Requirement	
12.4.2 Is distilled or deionized water used for final rinse?	Requirement	
12.4.3 Is an SOP available for glassware washing?	Requirement	
13 Purification and Slide Preparation Technician:		
13.1 What IMS kit/manufacturer is used?		
13.2 Is the supernatant from the centrifuged sample aspirated no lower than 5 mL above the pellet? [Section 13.2.2]	Requirement	
13.3 Is the pellet vortexed a sufficient time for resuspension? [Section 13.2.3]	Method Procedure	
13.4 Does the lab have an appropriate SOP for dividing pellets greater than 0.5mL into subsamples and analyzing?	Requirement	
13.5 Is no more than 0.5 mL of pellet used per IMS? [Section 13.2.4]	Method Procedure	
13.6 Is the leighton tube rotated at 18 rpm for 1 hour at room temperature?	Method Procedure	
13.7 Is the resuspended pellet volume quantitatively transferred to the Leighton tube (2 rinses)? [Section 13.3.2.1]	Method Procedure	
13.8 Are the IMS beads thoroughly resuspended prior to addition to the Leighton tube? [Section 13.3.2.2]	Method Procedure	
13.9 Is the sample quantitatively transferred from the Leighton tube to the microcentrifuge tube (2 rinses)? [Section 13.3.2.13]	Method Procedure	
13.10 Is standard NaOH (5 µL, 1N) and standard HCl (50 µL, 0.1N) used? [See note on pg 37]	Requirement	
13.11 Is sample vortexed vigorously for 50 seconds immediately after the addition of acid and 30 seconds after the sample has set for 10 minutes at room temperature? [Section 13.3.3]	Method Procedure	
13.12 Is a second dissociation performed? [Section 13.3.3.10]	Method Procedure	
13.13 When the second dissociation is performed, does the laboratory: (A) use a second slide, or (B) add the additional volume to the original slide?		Circle one: A B

	Item to be evaluated	Classification	Yes, No, NA or Unknown
13.14	Are the slides clearly labeled so they can be associated with the correct sample?	Requirement	
13.15	What type of slides are used?		
13.16	Is slide dried at a) room temperature or b) 35 to 42 C? [Section 13.3.3.12]		Circle one: A B
13.17	If the slide is warmed, is incubator or slide tray calibrated and labeled?	Requirement	
13.18	Does the laboratory have an acceptable SOP for sample purification?	Requirement	
13.19	Other than the issues noted in items 13.1 through 13.18 (if any) were sample purification and slide preparation performed successfully?		
14	**Sample staining** **Technician:**		
14.1	What staining kit/manufacturer is used?		
14.2	Is FITC stain applied according to manufacturer's directions?	Method Procedure	
14.3	Are positive and negative staining controls performed?	Requirement	
14.4	Are the direct labeling reagents applied properly? [Section 15.2.1]	Method Procedure	
14.5	Are the slides incubated in a humid chamber in the dark at room temperature for approximately 30 minutes or per manufacturer's directions? [Section 14.4]	Method Procedure	
14.6	Are the labeling reagents rinsed away properly after incubation, without disturbing the sample? [Section 14.5]	Method Procedure	
14.7	Was the working DAPI stain prepared the day it was used? [Section 7.7.2]	Method Procedure	
14.8	Is stock DAPI stored at 0 to 8°C in the dark? [Section 7.7.2]	Method Procedure	
14.9	Is the DAPI stain applied properly and allowed to stand for a minimum of 1 minute? [Section 14.6]	Method Procedure	
14.10	Is the DAPI stain rinsed away properly without disturbing the sample? [Section 14.7]	Method Procedure	
14.11	Is the mounting media applied properly?	Method Procedure	
	14.11.1 What type of mounting media is used?		
	14.11.2 Are all the edges of the cover slip sealed well with clear fingernail polish, unless Elvenol is used? [Section 14.9]	Method Procedure	
14.12	Are the finished slides stored in a humid chamber in the dark at 0 to 8°C (humid chamber not required for Evenol)? [Section 14.10]	Method Procedure	
14.13	Does the laboratory have an acceptable SOP for sample staining?	Requirement	
14.14	Other than the issues noted in items 14.2 through 14.13 (if any) was sample staining demonstrated successfully?		
15	**Microscope and Examination**		
15.1	Is microscope equipped with appropriate excitation and band pass filters for examining FITC labeled specimens? (Exciter filter - 450-490 nm, dichroic beam-splitting mirror - 510 nm, barrier or suppression filter: 515-520 nm)? [Section 6.9.2]	Requirement	
15.2	Is microscope is equipped with appropriate excitation and band pass filters for examining DAPI labeled specimens? (Exciter filter - 340-380 nm, dichroic beam-splitting mirror - 400 nm, barrier or suppression filter - 420 nm) [Section 6.9.3]	Requirement	
15.3	Does the microscope have HMO or DIC, objectives? [Section 6.9.1]	Requirement	
15.4	Is microscope operation easily changed from epifluorescence to DIC/HMO?	Recommendation	
15.5	Does the microscope have a 20 X scanning objective? [Section 6.9.1]	Requirement	
15.6	Does the microscope have a 100 X oil immersion objective? [Section 6.9.1]	Requirement	
15.7	Is the microscope equipped with an ocular micrometer? [Section 6.9.1]	Requirement	
15.8	Is a stage micrometer available to laboratory? [Section 10.3.5]	Requirement	
15.9	Is a calibration table for each objective located close to the microscope(s)? [Section 10.3.5]	Requirement	

Item to be evaluated	Classification	Yes, No, NA or Unknown
15.10 Does the wattage of the mercury lamp meet the microscope specifications?	Requirement	
15.11 Has the mercury bulb been used less than the maximum hours recommended by the manufacturer? [Section 10.3.2.11]	Recommendation	
15.12 Does the positive control contain *Cryptosporidium* oocysts at the appropriate fluorescence intensity for both FITC and DIC?	Requirement	
15.13 Does the laboratory have an acceptable SOP for sample examination?	Requirement	
15.14 Other than the issues noted for items 15.1 through 15.13 (if any) were other microscope or examination issues acceptable?		

Note: All section references in [ ] refer to Method 1623 April 2001

Initial Demonstration of Capability
Data Summary Form

Laboratory Name	Laboratory ID	

Method Information		
Which method was used? □ Method 1622 □ Method 1623		
Filter used:	Elution method:	Concentration method:
IMS kit used:	Staining kit used:	
Volume of water spiked (L):	Volume of water filtered (L):	

Initial Demonstration of Capability Summary Data

Sample	*Giardia* (not required)		*Cryptosporidium*		Equivalent Sample Volume Analyzed (to nearest 1/4 L)	Turbidity (NTU)
	Estimated No. of Cysts Spiked	No. of Cysts Detected	Estimated No. of Oocysts Spiked	No. of Oocysts Detected		
Method blank						
Spiked reagent water 1						
Spiked reagent water 2						
Spiked reagent water 3						
Spiked reagent water 4						
Mean recovery						
Precision (RSD)						
Matrix unspiked						
Matrix spike 1						
Matrix spike 2						
Mean recovery						
Precision (RPD)						

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